5-27-83

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

002859

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Timothy Gardner, Product Manager #17
Registration Division (TS-767)

THRU: Edwin R. Budd, Section Head
Section II, Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: Dermal Lesions in Laboratory Animals Following Exposure to Fluvalinate. Studies Conducted with Fluvalinate to Define Etiology. (Acc. No. 249604).
20954-EUP-19

TOX Chem. No. 934

Zoecon Corporation has conducted and submitted a series of studies to determine the etiology of skin lesions in laboratory animals following exposure to fluvalinate. Each of these studies has been reviewed and classified as core-supplementary. None-the-less, the studies when considered collectively indicated by the weight of their evidence that,

- the appearance of skin lesions in rats is a result of a topical dermal phenomena and not the result of a systemically induced effect and,

- that the experimental data developed for rats though not directly applicable to dogs (and mice), because of the argument of species difference, is supportive in drawing the same conclusion for dogs (and mice) as was concluded for rats.

One consequence of these studies and their conclusion has been to define the NOEL for the 90-day rat study. The NOEL for the 90-day rat study is now established for systemic effects as 3.0 mg/kg/day.

A second consequence of these studies has been to eliminate from consideration, in dogs, the effect of skin lesions in determining the NOEL. This leaves only the question of spleen weight as an unresolved issue in defining the NOEL in the 90 (and 180-day) day dog study. If the question of the spleen is resolved the 90-day NOEL could be established for dogs at 5.0 mg/kg/day.

Currently the 90-day dog study is being used to calculate the NOEL for tolerance purposes.

Conclusion:

1. Based upon the evidence available it is reasonable to conclude that the issue of skin lesion formation in rats (dogs and mice) has been satifactorily resolved.

2. The NOEL for the 90-day rat feeding study is now defined as 3.0 mg/kg/day (equvalent to 60.0 ppm of compound in the diet).

3. The NOEL in the dog study still needs to be defined but would be 5.0 mg/kg/day (equivalent to 200.0 ppm of compound in the diet) if the question of spleen weight is resolved.

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Over iew:

Dermal lesions have been observed in rats, mice and dogs administered fluvalinate in subchronic and chronic oral toxicity studies. Rats and mice received fluvalinate incorporated in ground laboratory chow whereas dogs received the test compound enclosed in a gelatin capsule which was administered by oral gavage. Dermal contact of medicated feed occurred with rodents due to the nature of the study (chronic or subchronic feeding study). Dermal exposure between dogs and technical fluvalinate is also suspected to have occurred through contact with the animal's vomitus and diarrhea (NOTE: the compound is a centrally acting emetic in dogs and also produces diarrhea, both responses were dose dependent.)

Specific studies were designed to determine the etiology of the lesions. Lesions in the dog were observed in/on the feet, genitals and neck area. Lesions in the rat were observed in/on the upper body primarily in/on the head and shoulder area and forelimbs.

Careful consideration was given to selection of an appropriate test model. The investigation focused on the albino rat rather than the dog as explained herein.

Experiments were designated to isolate systemic exposure (by the oral route) from dermal exposure to determine whether the fluvalinate exposure resulting in dermal lesions was systemic (by the oral route) or topical. This isolation of routes could easily be done for rats but not for dogs. Oral fluvalinate, is as was mentioned earlier, an emetic in dogs. Rodents on the other hand do not have the ability to vomit. If rats received a dose of compound delivered directly into the stomach it can only be absorbed or eliminated in the feces. In the dog administration by gavage led to systemic exposure and vomiting and diarrhea, which assuredly resulted in skin contamination with the compound as both the vomiting and diarrhea were profusive. The investigation therefore focused on the albino rat.

The submitted studies have been reviewed and classified as core-supplementary. However, when the studies are considered collectively the weight of evidence leads to the conclusion that the lesions which appear on the rats are secondary to a dermal contact phenomena and not the result of systemic toxicity resulting from oral or dermal absorbtion. On the basis of the data presented this reviewer agrees with the general conclusion that the lesions are secondary to a dermal contact with fluvalinate and also agrees with the specific conclusion(s) of each individual study.

Rationale for the Position that Skin Lesions in Rats Are the Result of a Topical Phenomena Secondary to Dermal Contact with Fluvalinate and Not the Result of a Systemic Response.

IRDC studies numbered 322-049 and 322-050 showed that when test animals were orally gavaged with test compound and strict procedures were followed as not to dermally contaminate the animal no skin lesions and no scratching was observed at anytime period even though their toenails were not clipped or surgically removed. This result gave a strong indication that skin lesion formation was not the result of systemic exposure by the oral route in rats. However, those animals that received the test compound in their feed developed skin lesions and were also observed scratching themselves. However, having shown that the oral route was not involved in skin lesion formation it was necessary to show that the lesions were or were not caused by a systemic response by way of dermal absorbtion of fluvalinate.

A radiolabeled dermal absorbtion study was conducted by the Biochemistry Dept. of Zoecon Corp. (and reported as study number 3760-1A-06-82). This study conducted on two rats (one restrained from being able to orally groom itself, and the second rat not being restrained from being able to groom itself orally) revealed that oral grooming removed nearly all the applied compound from the skin. This was shown by the presence of ca. 70% of the radiolabel in the urine and feces, 11% in/on the carcass (both internal and spread out on the exterior of the hair coat through grooming) and only 2% of the radiolabel remaining at the application site. However, the animal that was restrained (oral ingestion precluded) revealed that nearly 78% of the applied material remained at the application site, 3% in/on carcass and 1% in the urine and feces. (NOTE: recovery efficiency in both animals was 84 and 82 percent respectively). This experiment effectively removed dermal absorbtion and a resultant systemic response as a possible explanation for the presence of skin lesions.

Having effectively eliminated both the oral and dermal route as possible routes for the generation of skin lesions through a systemic effect attention was focused on the probability of a localized topical response phenomena to fluvalinate.

It was known from previously submitted dermal studies (see "one-liners, Cas. No. 934; $ADLD_{50}$ Doc. No. 001786; 21-Day Dermal Range Finding, Doc. No. 002256 and a 21-Day Dermal Rabbit, Doc. No. 002256) that minimal dermal irritation was observed under the test conditions (NOTE: these studies are conducted with an occlusive wrap at the site of application).

Studies were conducted in Japan to test the probability of lesion formation via a localized topical response phenomena. Fluvalinate was applied topically to the dorsal cervical area of the rat. This area was not accessible by hind foot or mouth. Animals were observed scratching as close to the application site as the hind foot could reach. Lesions were formed and were similar in nature and progression as observed in chronic feeding studies. Scab formation was observed outside the zone of application. Scabs were not formed at the actual zone of application.

It was already known that the compound was not a strong irritant nor did it produce allergic contact dermatitis. Additionally a systemic response was effectively eliminated by the previously reported oral gavage and dermal absorbtion studies. An itch sensation was suspected.

It was reported (references were provided in the document) that stimulation of C-fiber mechanoreceptors located within the epidermis is the source of perceived itch sensation in humans and the effective stimulus for reflexive scratching behavior. Changes in the chemical environment of these C-fiber nerve termini, as well as mechanical stimulation of mechanoreceptors effectively trigger firing of the nerve.

Two experiments were conducted in Japan which consisted of the parenteral administration of fluvalinate into the skin of rodents. One experiment consisted of an intradermal injection (stratum germinativum of the epidermis; free nerve endings monitoring pain are found here) of fluvalinate. This resulted in a lesion (scratch marks observed) essentially no different from lesions observed in the chronic feeding studies. However a subcutaneous injection led to immediate and transient grooming of the injection site but no lesions (abrasions/ulcerations) were observed. This latter study was replicated and scratching and lesion formation was observed. However, it was argued that in this latter experiment there was leakage of the technical upward into the epidermis as evidenced by brown spot formation of the injection site. (NOTE: the color of the technical material is amber). This reviewer believes this explanation is plausible.

It is therefore concluded that the lesion formation as observed in subchronic, and chronic feeding studies as well as the 28-day pilot studies included with this submission (IRDC studies #322-049, -050.) are due to a localized topical dermal phenomena, and that the severity of the lesions result in severe and adverse secondary effects of the test animal.

The issue of skin lesion formation in rats is therefore resolved.

Other conclusions derived from the submitted studies are summarized below:

- °The Sprague-Dawley rat is not uniquely sensitive to topical lesion formation as caused by fluvalinate.
- °Skin lesion production is not due to the presence of cyano moiety or to the primary acid metabolite, chloroanilino acid.
- °Skin lesions, like other biological effects result sole[illegible] from one specific isomer, the 2R S isomer.
- °There was no positive evidence for a phototoxicity mechanism for lesion formation.
- °Self-inflicted trauma (scratching) plays a crucial role, in exacerbation of an existing lesion and prevention of healing.
- °Similar dermal lesions were produced by fenvalerate.

Rationale for the Position that Skin Lesions in Dogs Are the Result of a Topical Phenomena Secondary to Dermal Contact with Fluvalinate.

Fluvalinate was administered in a gelatin capsule by oral gavage at doses of 2, 5, 15 and 50 mg/kg/day. Emesis and diarrhea were observed in all groups including controls. The control group and the two low dose groups did not differ as to frequency and severity of these responses. The two high dose groups, however showed a log dose response increase in both severity and frequency of emesis and diarrhea.

Localized lesions persisting (in some cases) for weeks were reported in all groups receiving the test article. Although a log-dose response was not generally evident, a compound-related effect appeared to be present.

One skin lesion was observed in the low dose group with 8, 3 and 6 lesions occurring at the next successive higher dose levels at the 90-day reading. Generally, the rate of lesion occurrence was one per dog. Lesions were primarily noted in the neck area or the hind paw. Irritation was generally noted in the area of the prepuce or the vulva. The number of lesions appeared to be nearly equally distributed between sexes. No lesions were recorded for controls.

It was reported that dogs with lesions appeared to be suffering from pruritus; they licked, scratched, or chewed the affected areas repeatedly. The pruritus appeared to be more severe shortly after dosing since dogs which were quiescent in the morning chewed and scratched themselves two to three hours after they were dosed. Bacterial cultures of lesions revealed the presence of either Staphylococcus aureus or B-hemolytic Streptococcus in 5 dogs.

The number of lesions observed at anytime period (0-180 days) was as follows:

| Group | Male | Female | Total |
|---|---|---|---|
| 1 | 0 | 0 | 0 |
| 2 | 0 | 1 | 1 |
| 3 | 2 | 5 | 7 |
| 4 | 2 | 3 | 5 |
| 5 | 4 | 4 | 8 |

It was previously concluded that the skin lesions were either the result of systemic toxicity or the direct result of direct topical exposure to the test article leading to itching, scratching and subsequent formation of lesions. Topical exposure may be considered through animal contact of its own emesis and feces which would litter and contaminate the animal itself or the cage floor.

It has already been concluded for rats that the skin lesion formation was due to a localized topical dermal phenomena which was a stimulation of C-fibers in the epidermis by fluvalinate which resulted in an itching leading to a scratching of such varied duration and intensity that progressively severe lesions (in some cases) were formed and wound healing (i.e. lesion healing) was either prevented or impaired for the experimental duration in many cases. However there were also occurrances when lesions healed spontaneously.

This Toxicology Branch reviewer believes that the experimental data developed for rats though not directly applicable to the dog, because of the argument of species difference, is support for drawing the same conclusion for dogs as was concluded for rats. The following general parallel observations for both species are noted:

- Lesions for both species were observed in the area of grooming,

- Both species were observed scratching the areas of normal grooming,

- For dogs contact with the test article with the hind feet from the cage floor through the presence of vomitus and feces and transfer to the neck area during grooming on a regular basis resulted at times in a progressive and protracted lesion formation and presence similar to that described for rats although the method of contact and/or transfer varied between species. It is pointed out here that rats eliminated 30% of parent compound in the feces. Dogs may have eliminated a comparable amount. Assuming that the average weight of the dog is 10 kilograms the following <u>total</u> amounts of parent technical would have been administered; respectively the <u>total</u> doses would be 500, 150, 50 and 20 milligrams per capsule per dose group. Additionally an unspecified amount of the dose would have been diluted in the stomach and upper intestinal contents and vomited out by the dog. It can therefore be seen that a goodly amount of parent technical could have been available for dermal contact. Additionally, it should also be kept in mind that the technical material in all likely-hood accumulated on the animal with time.

- The pattern of random lesion appearance and disappearance (and in rats reappearance) in dogs was in some cases similar to that observed for rats.

Conclusion:

It therefore appears reasonable to concluded that the lesions observed in the dog studies are due, as they are in rats, to a localized topical dermal phenomena and are not the result of a systemic response.

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002859

Subject: Pilot Study to Evaluate the Effects of Nail Clipping on the Appearance and Reversal of Skin Lesions During Dietary Administration of ZR-3210

and

Pilot Study to Evaluate Feasibility of Toe Clipping to Alleviate Skin Lesions which Occur During Dietary Administration of ZR-3210

Test Compound: Half-Resolved ZR-3210 Technical (fluvalinate) Run 23, Analysis No. 0281028
Purity: 93.1%

Accession Number: 249604

Testing Facility: International Research and Development Corp. (IRDC)

Study Numbers: 322-049 and 322-050

Testing Period: August 13, 1981 - September 10, 1981

Report Submitted to Sponsor: October 30, 1981

A. Objective:

These studies were designed to answer several questions concerning the skin lesions which appear during dietary administration of Half-Resolved ZR-3210 Technical to Charles River CD-1 rats.

Tests were designed to ascertain whether these lesions were due to a systemic effect of the test material or possibly to dermal exposure to the diet-test article mixture. Also evaluated was the possibility that the animals caused the lesions by self-mutilation with their hindfeet nails. Specific questions these studies were designed to assess include:

1. Would the lesions appear if the test material were administered by gavage?

2. Once a lesion was found could it be reversed by clipping the hindfeet toenails to the quick and keeping them clipped?

3. Could the hindfeet toenails be surgically removed with repeated success?

4. Would rats with clipped toenails or surgically removed toenails develop lesions and if so, how severe would they become?

B. Species Selection:

Previous studies have been run with the Charles River COBS® CD® rat using this test material. Much data regarding the skin lesions which appear during dietary administration of the compound has been obtained on this strain from this source. Males were used exclusively because this sex has been shown to be the more susceptable to the skin lesions.

C. Justification for Route of Administration:

From previous studies it is known that the skin lesions occur when fluvalinate is administered in the diet. In order to assess whether the lesions are due to a systemic effects or due to dermal exposure, the test material was also administered by gavage.

Materials and Methods:

Eighty two (82) 4 week old male Charles River CD rats were obtained from the Charles River breeding facilities of Portage, Michigan. Animals were acclimated to the facilities and initially housed 3 to a cage for 3 days and then 1 to a cage for the duration of the experiment. All animals were uniquely identified. Feed and water were available ad libitum. Rooms were controlled for temperature, humidity, and a 12 hour light dark cycle, (Note: clean protective clothing including disposable suits, gloves, plastic booties and face masks were worn by the trained personnel who conducted the study).

Animals were randomly assigned to 4 groups. Those animals not meeting the selection criteria were sacrificed and discarded prior to study initiation.

The following table shows the design of the 2 studies:

| Study Number | Group | Number of Animals at Administration | Route of Test Material Administered | Dosage (mg/kg day) | Condition of Toes |
|---|---|---|---|---|---|
| 322-049 | 1 | 15 | In diet | 30 | Hindfeet nails clipped prior to study initiation. |
| 322-049 | 2 | 30 | In diet | 30 | Normal at initiation; ultimately assigned to either have nails clipped or left alone. |
| 322-049 | 3 | 15 | Gavage | 30 | Normal |
| 322-050 | 4 | 10 | In diet | 30 | Toenails surgically removed. |

All studies were terminated after 4 weeks (i.e., 28 days).

Group 1:

These animals had their hindfeet toenails clipped "to the quick" on the first study day and every Monday and Thursday as needed.

Group 2:

The toenails of these animals at study initiation were normal. However, once any animal developed a lesion covering approximately 1.0 sq. cm. of body surface it was assigned to either a "no clip" or "clip" group. Alternating assignments were made to these two groups. The "no clip" group was left alone and the toenails allowed to grow in a normal fashion. Animals placed in the "clip" group had their toenails clipped following the same procedure as described with Group 1. All animals were assigned to the "clip" group by the 5th day of the third week thus allowing 9 days for evaluation of the procedure prior to termination.

Group 3:

The toenails of all these animals were not altered and remained in their natural state (i.e., no clipping and no surgery). This group was initially gavaged at various times throughout the day and then beginning early in study week 3 the animals were gavaged routinely at 9:AM.

Group 4:

These animals had their hindfeet toenails surgically removed prior to study initiation using two (2) different procedures. The decision to include these 10 animals in the study as a separate group was made by the sponsor. It was the reasoning of the sponsor that the procedures could be evaluated and some information concerning toe healing and nail regrowth as well as appearance and severity of lesions might be obtained even though recovery from surgery would not be complete prior to study initiation.

One procedure employed a modification of an electrocautery unit. The tips of the toes just proximal to the nail were removed from 3 anesthetized rats three days prior to study initiation using a surgical attachment to the electrocautery unit. Ferric subsulfate was used to reduce bleeding. These animals were then group housed on corn cob bedding until study initiation.

Two days prior to study initiation, 7 other rats were anesthetized and their hindfeet toenails (nails and distal phalanges) were surgically removed using a toenail clipper. An attempt was made to remove the distal phalanx and all of the nail. Ferric subsulphate was utilized to reduce bleeding and aid clotting. These animals were returned to individual wire mesh cages once they recovered from the anesthetic.

The test article was offered in the diet on a mg/kg/day basis to Groups 1, 2 and 4. The concentrations were recalculated and the diets mixed weekly. The half-resolved ZR-3210 was initially mixed with acetone and incorporated into ground basal laboratory diet and mixed in a Hobart blender. The resulting pre-mixes were added to additional laboratory diet and mixed to obtain the desired concentration.

Group 3 animals received the test material by gavage in a corn oil vehicle. The test concentration was prepared fresh on a weekly basis and administered on a mg/kg/day basis. Dose volume was kept constant at 4.0 ml/kg/day. Body weights were recalculated weekly.

Each rat was given a detailed physical exam once each day throughout the study. During days 5, 6 and 7 of study week 3, the animals in Group 3 were observed regularly post-dose in order to ascertain the time course of the reaction observed following gavage.

Moribundity and mortality were recorded on the day they were observed.

Body weights and food consumption was measured weekly.

Results:

All animals in Groups 1 and 2 survived the 4 week study whereas only 10 of 15 animals, dosed by gavage, survived (Group 3). All animals in Group 4 survived with the exception of the 3 animals which were deliberately sacrificed as a result of poor recovery from surgery. These 3 animals were sacrificed at the request of the study director on the second day of the study. Some of the toes appeared necrotic and others were infected. It was the opinion of the study director that the poor condition

of the feet would not allow a fair evaluation of the skin lesion formation. The toenails of these 3 animals were removed using electrocautery. Those animals of Group 4 that had their toenails removed with a clipper developed inflammed and occasionally infected toes but no necrosis was observed. These animals were placed on study before the feet had time to heal adequately. The continued presence of fresh blood on the animals indicated that the healing process at the site of surgery was impeded.

Group mean body weight gains for Groups 1, 2, 3 and 4 were 108%, 109%, 78% and 140%, respectively.

The values for group mean food consumption (g/rat/day) were generally comparable between all groups for all weeks with the exception of Group 3 (oral gavage) where values were substantially lower for the first week only.

The reported values for group mean compound consumption (g/kg/day) (dietary only) also appeared comparable for all groups for all periods.

The clinical signs (Group 3, gavage) reported included excessive salivation, hypersensitivity, red material around the eyes, labored breathing, decreased motor activity and unsteady gait. All these signs were previously observed and reported in other similar type studies. Signs beginning with salivation generally appeared at 1.0 hour post-dosing and began to disappear about 5 hours after dosing.

Skin lesions (dietary groups only) were defined as noted below and were seen primarily on the head and shoulder areas of the rats. Reference to other studies will show that the locus of the lesions was generally similar, as was the definition for categorizing the lesions.

| | |
|---|---|
| Abrasion: | A moist area, void of hair, where part of the skin has been scrapped away but where the involvement is confined to the surface. |
| Ulceration: | A moist area, void of hair, where the skin involvement is not confined to the surface layer and where muscle tissue may be involved. |
| Scabbing: | A dry, hard area on the surface of the skin, void of hair. |

The examination of the skin lesion data has revealed the following results.

Animals that received the test article by gavage (toenails left untreated) manifested no lesions or ulcerations at any time during the study. (Note: no individual animal data was included in the report for this group). These were the Group 3 animals.

Animals that had their toenails clipped prior to study initiation and regularly thereafter as needed (Group 1) showed a lesion incidence of 53% during the study and a lesion incidence of 27% by the end of the study. None of these animals developed an ulceration.

Animals that had their toenails removed surgically (Group 4 electrocautery or clipped) manifested a 29% (2/7) lesion incidence with no ulceraton during the study. However, at study termination only 14% (1/7) of the animals manifested a lesion. Three animals manifested toenail regrowth one of which showed the presence of a continuous large lesion for the last half of the study.

Group 2 animals received no pretreatment of toenails until after they had developed some identifiable lesion. Animals were then placed into one of two groups on a alternate basis. The first group (2a) received no treatment and the second group (2b) had their toenails clipped after the lesion developed. Those animals receiving no treatment showed an 85% lesion incidence (including 2 animals, 15%, which also developed ulcerations) at study termination, compared to the clipped group that had a 42% lesion incidence and no animals with ulcerations.

Additionally, the comparison of the data across, Groups 1, 2 and 4 indicated that in those cases where lesions were present, treatment of the toenails reduced both the number of lesions as well as the size of the lesions.

Discussion:

The examination of the data appears to indicate that dermal contact with the medicated feed results in skin lesions. This statement appears reasonable based on the observations that lesions were only observed in animals that received the test article in the diet. Animals receiving the test article by gavage showed no lesions. Toxic signs were present indicating absorbtion.

All animals receiving the test article in the diet generally showed a similar frequency of contact with the medicated feed as reflected in the values of grams of food consumed per rat per day. Compound consumption also appeared comparable for all dietary groups when measured in g/kg/day. All these groups (Groups 1, 2 and 4) manifested skin lesions. However, the data revealed that the degree to which the lesion was expressed was dependent upon the timing and type of toenail treatment. Based upon the data presented increasingly effective control of lesion development both quantitatively and qualitatively occurred in the following order Group 2b, 1 and Group 4. Therefore the results of the experiments indicate that toenail clipping does provide a means of controlling the development of lesions.

Additionally, it appears reasonable to say at this point that the lesions are not of systemic origin but of a topical origin. In expanding this line of reasoning we have taken verbatim the following from the report.

> In order for the animal to feed, he has to enter the food jar with his head and forelimbs. Toward the end of the week the diet level in the jar is low and sometimes it is necessary for the anterior half of the animal to enter the jar. Between the diet scattered by the rat and the edge of the jar lid rubbing on the animal, the skin on the anterior portion of the animal is repeatedly exposed to the diet-test article mixture. It appears that this dermal contact then causes the animal to scratch the exposed areas with his hindfeet and create the skin lesions observed.

This reviewer finds no strong reasons to disagree with this line of reasoning.

Additionally, it was also observed that toenail removal either by electrocautery or clipping needs further work. Some toenails became necrotic and the animals had to be destroyed. In other cases some nails were not clipped back far enough and did grow back.

It was also believed that a 2-3 week healing period prior to study initiation would be desireable.

Conclusions:

The following conclusions can be drawn from the evidence presented:

- Skin lesions do not appear to be the result of a systemic effect of the test article by the oral route.

° Skin lesions appear to be a result of topical contact with the medicated feed. The test article per se does not produce skin lesions (see also other dermal studies on file with the technical article), but appears to initiate a scratching, which over a period of time, results in abrasions leading to ulcerations and scab formation.

° Abraded and/or lacerated skin aggravates lesion formation by directly introducing the test article into the wound.

° Non-medicated feed did not produce skin lesions under the test conditions (gavage study).

° Surgical removal of toenails controls lesion development both in the number of lesions formed and the extent of the lesion formation.

Comment:

In view of the importance of the oral gavage study, we are requesting some representative evidence that this portion of the study has been carried out.

Classification: Supplementary for studies 322-049 and 322-050.

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Hazard Evaluation Division (TS-769)

Attachment:

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Fluvalinate Tox review 002859

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002859

Subject: Pilot Study to Examine Skin Lesion Etiology.

Test Compound: Half-Resolved ZR-3210 Technical (Fluvalinate).
Run 23, Analysis No. 0281028
Purity 93.1%

Accession Number: 249604

Testing Facility: International Research
and Development Corporation

Study Number: 322-051

Testing Period: November 19, 1981 - December 17, 1981

Report Submitted to Sponsor: March 8, 1982

A. Objective:

This study was designed to further study the cause of the skin lesions which appear during dietary administration of Half-Resolved Fluvalinate Technical to Charles River CD rats. Two other strains of rats the Fischer 344 and the Long Evans, were simultaneously tested in order to discriminate the relative susceptibility of these two strains to the formation of these lesions. Along with this, additional Charles River CD rats were fed a pelletized form of diet containing Half-Resolved Fluvalinate to see if this might reduce exposure and thus have an effect on the formation of the lesions and finally three groups of Charles River CD rats were dusted with 1) ground Purina® Chow #5002 2) ground Purina® Chow #5002 mixed with Fluvalinate and 3) cornstarch mixed with Fluvalinate while they all received untreated ground Purina® Chow #5002 as diet. If dermal exposure alone were responsible for the irritation which stimulates the animals to create the lesions, these dermally exposed groups should confirm this hypothesis.

B. Species Selection:

Previous studies have been run with the Charles River CD® rat using this test material. Considerable data regarding the skin lesions which appear during dietary administration of the compound have been obtained on the CD strain from this source. Males were used exclusively because this sex has been shown to be the most susceptible to the skin lesions. Two other strains were selected (Fischer 344 and Long Evans) to see whether these strains also developed skin lesions upon dietary administration of test material.

C. Justification for Route of Administration:

From previous studies it is known that the skin lesions occur when 30 mg/kg/day fluvalinate is administered to Charles River CD rats in the diet. Test material was administered in this manner to three strains of rats for comparison purposes. Also, previous studies indicated a possible need for dermal exposure to the compound before skin lesions were formed. Therefore mixtures of diet or cornstarch with fluvalinate were sprinkled on to the fur of the animals once a day in order to obtain dermal exposure with the least possible oral exposure.

Another group received pelletized diet/test article mixture with the expectation that pelletization would reduce the dermal exposure to the test article.

Materials and Methods:

Ninety-nine male Charles River CD rats, 22 male Long Evans rats and 23 male Fischer 344 rats, all 28 days old, were obtained from the Charles River Laboratories of Portage, Michigan. Each strain was housed separately, one animal to a cage, during the 2 week acclimation period and actual test period. The animal's environment was controlled for temperature, humidity and a 12 hour light/dark cycle. Food and water were available ad libitum. Each animal was identified by a metal ear tag carrying a unique number.

Clean protective clothing including disposable suits, gloves, plastic booties and face masks were worn by the trained personnel who conducted the study.

The animals were randomized into study groups by weight 1 week prior to study initiation. The animals from Group 1 were placed on pelletized untreated Certified Purina® Rat Chow #5002 1 week prior to study initiation in order that they become accustomed to eating the diet in this form.

The following table shows the design of the study:

| Group | Number Animals | Method of Dosing | Test Material Carrier | Dosage of ZR-3210 | Species/Sex of Rat |
|---|---|---|---|---|---|
| 1 | 15 | Dietary | Pelletized Purina #5002 | 30 mg/kg/day | CD/Male |
| 2 | 15 | Dermal | Powdered Purina #5002 | 0 (Acetone Control) | CD/Male |
| 3 | 15 | Dermal | Powdered Purina #5002 | 600 ppm | CD/Male |
| 4 | 15 | Dermal | Cornstarch | 600 ppm | CD/Male |
| 5 | 15 | Dietary | Ground Purina #5002 | 30 mg/kg/day | CD/Male |
| 6 | 15 | Dietary | Ground Purina #5002 | 30 mg/kg/day | Fischer 344/Male |
| 7 | 15 | Dietary | Ground Purina #5002 | 30 mg/kg/day | Long-Evans /Male |

All treated diets for Group 1 were mixed at IRDC using predicted (historical) food consumption and body weight values and sent to Zoecon Corp. for pelletization (Note: pelletization required the use of a dextrose binder which comprised 10% of the total weight of the end product). Concentrations were adjusted weekly and pelletized diets were kept frozen until ready for use.

In Group 3 and 4, the test article was mixed with either basal laboratory diet which had been milled extremely fine or cornstarch and applied dermally. To do this, about 1/4 teaspoonful of the mixture was sprinkled on the head, shoulders and back region (anterior end only) of each animal daily. This amounted to about .6 g of basal laboratory diet-test article mixtures and .8 g of cornstarch-test article mixture. Then the fur was ruffled gently with a gloved hand in order that the mixture fall to the surface of the skin. This same procedure was used for Group 2 where untreated milled basal laboratory diet was applied.

The rat chow to be used for dermal application was powdered at Zoecon and sent to IRDC for use.

The diets with test material used for Groups 5, 6, and 7 were prepared weekly at IRDC using the previous week's food consumption and body weight data.

Animals were observed daily for signs of toxicity and for the appearance of skin lesions. A careful record of each lesion was kept indicating location, size and severity. Lesions were categorized as follows:

Hairloss: Area void of hair, surface of skin exposed.

Abrasion: A moist area, void of hair, where part of the skin has been scraped away but where the involvement is confined to the surface.

Ulceration: A moist area, void of hair, where the skin involvement is not confined to the surface layer and where muscle tissue may be involved.

Scabbing: A dry, hard area on the surface of th skin

Moribundity and mortality were recorded on the day they were observed.

Individual body weights were measured and recorded weekly beginning one week prior to study initiation.

Individual food consumption values were measured and recorded weekly beginning one week prior to study initiation.

All statistical analysis compared the treatment groups with the control group for body weight gain and food consumption.

Results:

Clinical Signs: It was reported that one animal receiving the test article in the diet manifested excessive salivation. It was also reported that neck swellings were noted in many animals in all test groups. This swelling was attributed to a viral infection causing an enlargement of the salivary glands in the region of the neck. This observation has been reported in other experiments and the explanation is plausible.

Mortality: No animals died during the course of the study.

Mean Body Weights: Mean body weights for Group 1 (pelletized feed) and Group 6 (dietary, Fischer 344) were statistically significantly lower than control values at termination. However, the initial mean body weights for these two groups were substantially lower than control (Group 2, acetone in basal diet) values at the beginning of the study. The significance is therefore judged to be not biologically meaningful. All other group mean body weights were comparable to the control group value.

Mean Food and Compound Consumption: Values were not statistically different between treated groups and control group with one exception. Group 6 animals (Fischer 344) showed a statistically significant decrease in grams food/animal/ consumed per day. However, on a grams per kilogram basis and a compound per kilogram basis this group was comparable to the control group. The decrease in grams food/animal/consumed per day appears to reflect the original small stature of this group (Note: the original mean body weights of Group 6 were even smaller than Group 2 by almost 40 grams).

A summary of the skin lesion data is presented in tabular form (Attachment #1) and reflects the individual skin lesion data. No ulcerations were observed, only abrasions and scabbing.

Discussion:

Examination of the data revealed generally similar responses for all categories of observation for Groups 1, 5, 6 and 7, with the exception of Group 6 which had a low incidence of severe lesions (lesions were considered severe if the abraded area exceeded 2.0 sq. cm.) and a low incidence of the number of animals with lesions at the end of the study. The incidence and severity of lesions in these groups were also higher than those observed in Groups 2, 3 and 4 (dermal dusting). Groups 2, 3, and 4 also revealed generally similar responses for all categories of observation. No animals in these three groups developed severe lesions. It was also noted that because the test article mixture was dusted on the head and shoulders of the animals in Groups 3 and 4, some oral ingestion equivalent to about 2.4 mg/kg/day could not be denied. The contractor therefore put forth the argument that an animals highly sensitive to the test article might have responded to this dose (2.4 mg/kg/day) and thus a few dermally dusted animals developed lesions as a result of the oral exposure. However, it was pointed out by the contractor, and now by this reviewer, that in a previously conducted study when fluvalinate was administered by oral-gavage at a dose of 30 mg/kg/day for 28 days, no dermal lesions were observed.

The contractor concluded that within the confines of this study (IRDC Study No. 322-051) it appeared that both a dermal and systemic factor may have been involved in causing skin lesion formation and suggested further studies to explain the cause of the lesions.

However, based upon the results of this study and previous studies (IRDC Study No. 322-049 and 322-059) the oral route (i.e., oral ingestion leading to systemic effects as a cause of lesion formation) appears less likely as a route for lesion formation.

The following question has been raised in the course of the review, "what is meant by the phrase variation in feeding days" (pp. 122, 123).

Classification: Supplementary.

Albin B. Kocialski

Albin B. Kocialski, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769)

Attachment

OPP:HED:TOX:A. KOCIALSKI:sb 4/6/83 X77395 Rm 820 #m26

| Group | Treatment | Concentration or Dose | Total No. Animals | Strain | Day of Appearance of First Lesion | No. Animal Developing Lesions (%) | No. Animals with Lesions in Places Other than Around Eartag(%) | No. Animals Developing Severe Lesions (%) | No. Anima With Lesi At End of Study ( |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Pelletized Diet | 30 mg/kg/day | 15 | CD | 2 | 15 (100) | 14 (93) | 5 (34) | 14 (93) |
| 2 | Dermal - Control Diet | Acetone Control | 15 | CD | 11 | 4 (27) | 2 (13) | 0 (0) | 0 (0) |
| 3 | Dermal - in Diet | 600 ppm | 15 | CD | 9 | 3 (20) | 0 (0) | 0 (0) | 3 (20) |
| 4 | Dermal - in Cornstarch | 600 ppm | 15 | CD | 2 | 3 (20) | 1 (7) | 0 (0) | 1 (7) |
| 5 | Oral | 30 mg/kg/day | 15 | CD | 3 | 15 (100) | 14 (93) | 6 (40) | 13 (87) |
| 6 | Oral | 30 mg/kg/day | 15 | Fischer 344 | 2 | 13 (87) | 10 (67) | 1 (7) | 7 (47) |
| 7 | Oral | 30 mg/kg/day | 15 | Long Evans | 5 | 13 (87) | 12 (80) | 6 (40) | 10 (67) |

#m2

Subject: Pilot Study to Develop Procedure for Toenail Removal and Evaluate the Effect on Skin Lesion Formation in Rats.

Test Compound: Half-Resolved ZR-3210 Technical (Fluvalinate). Run 23, Analysis No. 0281028. Purity 93.1%

Accession No.: 249604

Testing Facility: International Research and Development Corp.

Study No.: IRDC No. 322-052

Testing Period: December 3, 1981 - December 17, 1981

Report Submitted to Sponsor: February 24, 1982

A. Objective

This study was designed to evaluate the effect of toenail removal on the appearance of skin lesions in Charles River CD® rats during dietary administration of fluvalinate.

B. Species Selection

Species selection has been previously described in IRDC reports numbered 322-49, -50, -51 of this accession (Accession#249604).

C. Justification For Route of Administration

Justification has previously been provided in IRDC reports numbered 322-49, -50, -51 of this accession (Accession#249604).

Materials and Methods:

Forty-three (43) three week old male Charles River CD rats with the nails of all toes on all feet removed were obtained from Charles River Kingston, Stoneridge, New York. Nails were removed just proximal to the nail bed by the shipper. The animals were 18 days old when they were denailed and 38 days old when the study was initiated (NOTE: 20 days were allowed for healing but eventually was shown not be sufficient). All rats were housed individually in an environmentally controlled room. Food and water were available ad libitum. Each animal was identified by a metal ear tag carrying a unique numeral.

Clean protective clothing including disposable suits gloves, plastic booties and face masks were worn by trained personnel who conducted the study.

The design of the study was as follows:

| Group | Number of Animals | Dosage of Fluvalinate |
|---|---|---|
| 1 | 21 | 0 (control) |
| 2 | 21 | 30 mg/kg/day (in feed) |

Animals were assigned to either group using a random number generator.

The test article was offered on a mg/kg/day basis. The concentrations were recalculated and the diets mixed weekly.

A diet pre-mix composed of test article, acetone and basal laboratory rodent chow was added to ground basal laboratory diet to attain the proper concentration and blended in a twin-shell Hobart blender.

All animals were observed over a two (2) week period for appearance, behavior, mortality, moribundity, body weight change and food consumption.

Results:

Mortality: No animals died while on the study.

Body weight gain and food consumption was greater for controls than treated groups. The control group gained 30 grams more than the treated group and ate 5.0 grams more per animal per day than the treated group. General observations were not remarkable (skin lesions considered separately) with the exception of corneal opacities in 10 animals which were present prior to study initiation. The condition persisted unchanged throughout the study. The corneal opacities were attributed to a viral agent. The condition of toes upon arrival for all rats showed swollen feet with scabs on all toes. It was reported by the contractor that as the study progressed white, bony protrusions were observed on the tips of some toes. Both the scabs and bony protrusions dropped off and the toes healed completely in some cases. Some

of the animals had scabs and/or bony protrusions at study termination. The contractor for the purpose of compilation of data, considered toes to be healed when all scabs had disappeared from the tips of the toes. It was reported that in the majority of animals, complete healing of most or all toes took place during the course of the study. The number of animals with completely healed toes by study termination were 15/21 in the control group and 11/21 in the treated groups. Therefore 6 animals in the control group and 10 animals in the treated group did not have completely healed toes by the end of the experiment.

Skin lesion formation was not observed in the control group. Skin lesion formation was observed in 14 of 21 treated animals with 3 animals manifesting a severe lesion (area greater than 2.0 sq. cm.)

Discussion and Conclusion:

The data indicated that 14 animals in the treated group developed skin lesions after study initiation whereas none were observed in the control group. However, it was not readily apparent as to how these lesions were formed as all the animals in both groups had all their toenails removed. It was also interesting to note that, as reported by the contractor, that the location of the lesions were primarily ventral cervical as opposed to dorsal cranial and cervical in rats who did not have the toenails removed in other experiments. It was speculated that these lesions which were all located in/on the head and shoulders may have resulted from the animals rubbing against the rim of the food jar.

It was concluded by the contractor that removing the toenails from the rats prior to initiation of dosing with the treated diet did not eliminate the formation of skin lesions in Charles River CD rats. Since lesions were not prevented using surgical declawing, this procedure was found not acceptable as a procedural option to be used in the treatment of rats placed on a chronic feeding study.

Classification: Supplementary

Gilbert B. Kocialski

TS-769:th:TOX/HED:ABKocialski:4-11-83:card misc. #27

Subject: 14-Day Rat Dietary Study with Fluvalinate-R-S (Fully Resolved, i.e. one isomer).

Test Compound: Fluvalinate-R-S (Fully resolved).
RU 40074 Lot 2 ZPA No. 1611.
Purity: 98.4%

Accession No: 249604

Testing Facility: IRDC

Study Number: 322-054

Testing Period: February 4 - February 18, 1982.

Report Submitted to Sponsor: April 6, 1982

Objective:

The objective of this study was to evaluate the toxicity of fully resolved Fluvalinate-R-S to rats upon repeated dietary administration and to evaluate the potential to produce skin lesions.

Species Selection:

This species was also used in other studies, and was selected for this study for comparison purposes.

Materials and Methods:

Twenty-two (22) male Charles River CD rats (28 days old) were obtained from the Charles River Breeding Laboratories Inc., Portage, Michigan. All animals were housed individually in suspended wire mesh cages in an environmentally controlled room. Food and water were available ad libitum. Animals were acclimated for 14 days to laboratory conditions and examined twice daily for physical and pharmacological/toxicological changes. Following the 14 day acclimation period 15 animals free of physical abnormalities and in good health were randomly selected and assigned to the treatment group. A matching control group was not employed. All animals on study were uniquely identified.

Test article was presented in the basal laboratory diet (Certified Rodent Chow® #5002, Purina). The test article was dissolved in acetone and added to 500g of chow which formed the pre-mix. Additional laboratory chow was added to the pre-mix to obtain the desired final concentration followed by blending in a Hobart mixer. The test article was offered in the diet at concentrations estimated to achieve a dosage level of 15 mg/kg/day of active ingredient. (NOTE: This concentration of 15 mg/kg/day is equivalent to 30 mg/kg/day of the half resolved). The concentration of the test article in the diet was adjusted each week according to the most recent body weight and food consumption measurements.

Trained personnel conducting the study wore disposable plastic protective clothing.

The animals were observed twice daily, seven days a week for signs of overt toxicity, moribundity, mortality and skin lesions. Skin lesions were categorized as follows:

Hair loss:

Area void of hair, surface of skin exposed.

Abrasion:

A moist area, void of hair, where part of the skin has been scraped away but where the involvement is confined to the surface.

Ulceration:

A moist area, void of hair, where the skin involvement is not confined to the surface layer and where muscle tissue may be involved.

Scabbing:

A dry, hard area on the surface of the skin void of hair.

002859

Body weights were recorded weekly.

Food consumption was measured prior to study initiation and after 3, 7 and 14 days.

Results:

All animals survived the study and showed a 27% mean body weight gain. Mean weight gain was accompanied by an increased mean food consumption of about 25% per gram per rat per day.

Skin lesions were noted for all animals on the study. Scabbed areas were noted for all but one rat with the primary location being the upper body. Lesions were also observed on the forelimb(s). Abraded areas were primarily located in the upper body (head, neck, shoulder). Hair loss was also observed on the head, shoulder and forelimbs for all but 4 rats on the study.

Lesions persisted on almost all animals till termination of the study. Systemic signs were verbally reported to be the same as those seen with the half resolved technical at 30 mg/kg, especially copious salivation.

Discussion:

Fluvalinate, fully resolved technical (15 mg/kg/day), produced lesions comparable to lesions previously seen in other studies with the half resolved technical. Lesion incidence, location (and severity as stated by the registrant) were reported to be highly similar to that seen previously with the half-resolved at 30 mg/kg/day and the un-resolved technical at 60 mg/kg/day. The author stated and this reviewer agrees that the results appear to be consistent with prior evidence that the biological activity is limited to R-S isomer.

Classification: Supplementary.

(NOTE: the unresolved technical contained 4 stereoisomers the half-resolved technical contained 2 stereoisomers and the fully resolved technical contained one stereoisomer which is the biologically active isomer).

Albin B. Kocialski

Albin B. Kocialski, Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769)

TS-769:KOCIALSKI:sll:X557-7394:3/26/93 Rm. 820 #m24

Subject: 14-Day Rat Dietary Study with R-N-(2-chloro-4-trifluoromethylphenyl valine)

Test Compound: R-N-(2-chloro-4-trifluoromethylphenyl valine). 514-37. ZPA No. 1612. Anal. No. 0182015.
Purity: 99.6%

Accession No.: 249604

Testing Facility: IRDC

Study No.: 322-055

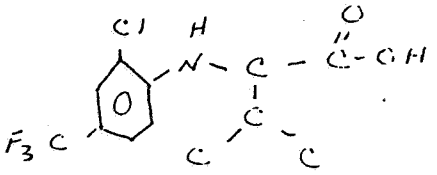
Testing Period: February 4-18, 1982

Report Submitted to Sponsor: April 6, 1982

Objective:

The objective of this study was to evaluate the toxicity of R-N-(2-chloro-4-trifluoromethylphenyl valine).

Structure:



Species Selection:

This species was also used in other studies and was selected for this study for comparison purposes.

Materials and Methods:

Twenty-one (21) male Charles Rvier CD rats were obtained from the Charles River Breeding Laboratories of Portage, Michigan. Animals were housed individually in an environmentally controlled room for 14 days of acclimation. Food (basal laboratory diet, Purina) and water were available ad libitum. Animals were observed twice daily for signs of overt toxicity, and mortality. The condition of the haircoat was recorded daily. Following the acclimation period 15 males with no apparent physical abnormalities were selected randomly and assigned to the treatment group. Each animal received a unique identifying number.

The test article was offered in the diet at a concentration estimated to achieve a dosage level of 18 mg/kg/day (molar equivalent of 30 mg/kg of half-resolved, assuming 100% hydrolysis of the latter). The test article was incorporated into the feed using procedures previously described (see IRDC study nos. 322-049, and 322-050). The test article concentration was adjusted weekly using the previous weeks body weight and food consumption values.

The rats were observed twice daily, seven days a week for signs of overt toxicity, moribundity and mortality. Detailed physical examinations were recorded daily for general appearance and behavior, signs of toxicity and for the appearance of skin lesions.

In the event that skin lesions occurred during the study, a careful record of the lesion was to be recorded including the location, size and severity. Any lesion present was to be categorized as follows:

Hair loss: Area void of hair, surface of skin exposed.

Abrasion: A moist area, void of hair, where part of the skin has been scraped away but where the involvement is confined to the surface.

Ulceration: A moist area, void of hair, where the skin involvement is not confined to the surface layer and where muscle tissue may be involved.

Scabbing: A dry, hard area on the surface of the skin void of hair.

Body weights and food consumption values were recorded weekly.

Results:

It was reported that no animlas died during the study and that appearance and behavior showed no signs of a compound-related effect. It was reported that one animal exhibited hair loss (1 day duration, day 3) in the neck region but that the finding was considered incidental. We agree. Average compound consumption was 20 mg/kg/day and mean food consumption increased from a pre-test value of 23.8 g/rat/day to 28.9 g/rat/day by the end of the 2 week observation period. The group mean body weight gain from day 0 to day 14 was 50%.

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Discussion and Conclusion:

This reviewer agrees with the sponsor's conclusion that when R-N-(2-chloro-4-trifluoromethylphenyl valine) was offered in the diet to Charles River CD rats for 2 weeks at a dosage level of 18 mg/kg/day, no treatment-related effects were observed.

Classification: Supplementary

Albin B. Kocialski, Ph.D.
Toxicology Branch/HED (TS-769)

Albin B. Kocialski

TS-769:th:TOX/HED:ABKocialski:4-11-83:card misc. #27

002859

Subject: Pilot Study to Evaluate the Effect of Corn Oil Gavage Dosing, Vehicle Volume on the Toxicity of Fluvalinate.

Test Compound: Fluvalinate

Accession No.: 249604

Testing Facility: IRDC

Study No.: (0219E)

Testing Period: January/February 1982

Writeup Submitted by Sponsor: Ca. February 1982

NOTE: This was an informal experiment conducted by IRDC as a favor to Zoecon to aid in finalizing the chronic study (daily gavage dosing) design. There was no written protocol and no written report was prepared.

Materials and Methods: Two high doses of fluvalinate (technical) [20 and 30 mg/kg/day) were delivered each at 3 different corn oil dosing volumes (1.0, 2.5 and 5.0 ml/kg) to

6 groups of 10 Charles River D weaning male rats. Animals were observed daily for mortality and toxic signs for a period of 21 days.

Results: (Telephone reports were provided weekly). During the first week, 30 mg/kg produced distinctly more severe toxic signs than did 20 mg/kg and high dose volumes began to be observed to be associated with more severe toxic signs than low dose volumes. Onset of signs became progressively later with respect to the time of daily dosing. Additionally, the duration of signs following administration of daily dose diminished as the study progressed. The most severely affected animals were still showing toxic signs 24 hours post-dosing. The least affected group was the 20 mg/kg group dosed at 1.0 ml/kg.

The only compound-related deaths occurred in the high dose group with 3/10 and 2/10 animals dying at dosing volumes of 5.0 and 2.5 mls/kg., respectively.

There were notably more toxic signs at 30 than at 20 mg/kg and at higher dose volumes. The relative severity of toxic signs increased with dose and increased with the volume administered with dose.

It is also pointed out that one of the signs observed was an abnormal gait characterized by the animal walking raised up walking with weight on proximal phalanges and metatarsals and the tail rigidly extended.

Body weight and feed consumption showed little difference between groups.

No dermal lesions occurred in any of the 60 animals on test even at dose levels and volumes causing death and marked systemic toxic signs. These findings reinforced prior test indications that previously seen dermal lesions did not result from administration of fluvalinate by the oral route.

Conclusion:

Based on the data, it was determined that 20 mg/kg could safely be administered as the top dose on a repeated daily dosing gavage study provided the dose volume was low. On this basis, a constant.1 ml/kg corn oil dose volume was selected for the chronic study.

Classification: Supplementary

Albin B Kocialski

Albin B. Kocialski Ph.D.

Toxicology Branch/HED (TS-769)

37

002859

Subject: Pilot Study to Evaluate the Effect of Daily Topical Administration of Fluvalinate in Two Vehicles and of Corn Oil Alone.

Test Compounds: See Material and Methods Section.

Accession No.: 249604

Testing Facility: IRDC

Study No.: (0185E)

Testing Period: January 1983

Write up Submitted by Sponsor: January 1983.

NOTE: This was an informal experiment conducted by IRDC as a favor to Zoecon to aid decisions regarding the need for changes in methodology on the ongoing chronic study. There was no written protocol and no written report was prepared.

The two page report has been xeroxed in its entirety and is attached.

Classification: Supplementary

Albin B Kocialski

Albin B. Kocialski, Ph.D.

Toxicology Branch/HED (TS-769)

DCR-17396:Kocialski:TOX-16:4/19/83:CM#2:Rm820:x77395:efs

REVISED:04/20/83:DCR-18048

Fluvalinate Tox review 002859

Page_____ is not included in this copy.

Pages 40 through 41 are not included.

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X FIFRA registration data.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Subject: Toxicity of Fluvalinate to Rats: Fluvalinate vs. ZR-3159 (Acyanofluvalinate).

Test Compound: Fluvalinate half-resolved (92.6% pure with 4.0% m-phenoxybenzaldehyde and less than 1.0% R-Chloroanilino acid) and Acyanofluvalinate (96% pure).

Accession No.: 249604

Testing Facility: Biochemistry Dept., Zoecon Corp., Palo Alto, California.

Report No.: 7270-1A-02-82

Testing Period: February 22 - March 15, 1982.

Report Submitted: April 12, 1982.

Materials and Methods: Five week old male Simonsen albino (Sprague-Dawley-derived) rats were obtained from the Simonsen Laboratories of Gilroy, California. Animals were housed 4 to a cage for a 5 day acclimation period under standard laboratory conditions. Six days after arrival 10 animals were randomly assigned to two groups, individually housed, and had their

body weight recorded. Animals were not uniquely identified, however never was more than one animal removed from a cage at the same time. The experimental design was as follows:

| Group | Number of Animals | Route of Administration | Target Dose mg/kg/day | Test material |
|---|---|---|---|---|
| A | 5 | in diet | -30 | fluvalinate |
| B | 5 | in diet | -30 | ZR-3159 |

Animals were observed periodically (except Saturdays and Sundays) for general appearance and behavior. Animals were also observed for skin lesions using the same criteria as described previously in other reports (see this accession, IRDC reports 322-049 and 322-050).

Body weights and food and water consumption were measured weekly. Food (S/L Custom Lab Diet #4.5 Simonsen Labs.) and water were available ad libitum.

Additionally, at the end of the 3-week test period animals were placed on a lab diet devoid of the test article and observed for 1 week for healing and/or reversal of any occurring skin lesions.

The test compound was provided in the diet on a ppm basis with the concentration, recalculated and mixed weekly. Test article incorporation into the basal diet generally followed procedures described previously for fluvalinate in other subchronic and chronic feeding studies.

Results: An unusual jerking motion was observed in both groups as well as a stretching movement not related to waking from sleep. Periodic erratic movements were observed including jumping in and out of the food dish, biting of the toes, scratching and a digging action in the bedding. One animal in Group A was observed salivating and scratching itself under the skin. Diarrhea was also observed in most animals of both groups.

Skin lesions were observed in both groups. Group A, however, manifested an 80% lesion incidence whereas Group B showed only a 40% lesion incidence. Group A showed ulcerations in 4/5 animals at termination whereas, Group B had only 1/5

animals with an ulceration. The majority of the lesions were located on top of the head, behind the ears or shoulder area.

Food and water consumption values were difficult to interpret due to urinary and fecal contamination of some feeders, and technician oversight. However, body weight gains for both groups paralled one another for the 3 weeks (+12%; +34% +50%).

Discussion and Conclusion: Skin lesions were observed in both groups although to a greater extent and severity in Group A. The locus of the lesions was, however, generally similar in both groups.

We agree with the conclusions of the sponsor that in the absence of a control group and the small size it is difficult to assess definitively the contribution of the cyano group in lesion formation. However, on a preliminary basis under the described test conditions one might say that the presence of the cyano group does not appear to be necessary to the formation of skin lesions.

It was also stated that after 3 weeks on the test article diet rats were provided untreated feed and lesion recovery was observed. However, the original report contains no record of these observations.

Classification: Supplementary.

Albin B. Kocialski Ph.D.
Toxicology Branch/HED (TS-769)

Subject: Metabolism of $C^{14}$ Fluvalinate Applied Dermally on Rats.

Test Compound: Carbon-14 radiolabeled fluvalinate (Purity ca. 93%)

Accession No.: 249604

Testing Facility: Biochemistry Dept. of Zoecon Corp., Palo Alto, California

Report No.: 3760-1A-06-82

Testing Period: October 1982

Report Written: October 1982

Materials and Methods:

Two (2) male Sprague-Dawley rats (182 and 219 grams; 7 weeks old) were prepared one day prior to treatment by shaving an area on the mid-dorsal region approximately 4 sq. cm. One animal was equipped with a harnesslike restrainer which prevented access to the shaved area by mouth or feet but yet allowed the rat to walk. No irritation was visible around the shaved areas of either rat.

Each rat was treated with [trifluoromethyl-$C^{14}$] fluvalinate (3.7 mg, 29 u Ci, 3.73 u Ci/m mole) in acetone (50 ul) by dripping the solution on the shaved area via syringe. Immediately after dosing each animal was maintained in an all-glass metabolism chamber for separate collection of urine and feces.

Within 15-20 minutes the unrestrained rat began thoroughly grooming the treated area and within 2 hours was exhibiting toxic signs (salivation, retching). The animal appeared normal after four hours.

Immediately after dosing the restrained rat showed signs of severe discomfort by writhing extensively.

Urine and feces were collected for 4 days and then analyzed.

Animals were sacrificed with ether after 4 days.

The treated skin was excised, the exterior surface rinsed with acetone and an aliquot of the rinse quantified by liquid scintillation counting (LSC). The skin patch was then extracted with methanol and an aliquot of the filtrate was quantified by LSC. Unextractable $C^{14}$ residues and radioactivity in the minced carcass remains were quantified by combustion to carbon dioxide labeled $C^{14}$.

Results:

Recovery of the radiolabel as a percent of the applied dose from rats treated dermally with [trifluoromethyl-$C^{14}$] fluvalinate was as follows:

| | Restrained | Unrestrained |
|---|---|---|
| Urine (Total) | 0.7 | 10.8 |
| Feces (Total) | 0.8 | 59.7 |
| Skin patch (Total) | 78.3 | 2.3 |
| Carcass | 2.6 | 10.6 |
| Total Recovery | 82.4 | 83.4 |

The identity of the radiolabeled metabolites in the feces as a percent of the total radioactivity found in the feces was as follows.

| | Restrained | Unrestrained* |
|---|---|---|
| fluvalinate | 5.2 | 31.8 |
| anilino acid | 32.2 | 24.5 |
| polar products | 52.4 | 30.4 |

*(i.e. 60% of the applied dose that was found in the feces had a metabolite distribution as noted).

Discussion and Conclusion:

The examination of data for the restrained rat and the unrestrained rat revealed comparative total recovery efficiencies of 82.4 and 83.4 percent respectively. The restrained rat had 78% of the original dose remaining on the skin after 4 days. This indicated very little absorbtion of fluvalinate through the skin and was reaffirmed by the less than 2% radioactivity recorded for both urine and feces. The unrestrained animal showed little total radioactivity on the skin but a large combined count for both urine and feces. This reflected oral ingestion of material through grooming. In the unrestrained animal identifiable metabolites in the feces showed a roughly equal distribution between fluvalinate (31%), anilino acid (25%) and unidentified polar products (30%).

Additional examination of the data for the unrestrained rat revealed that of the total amount of radiolabeled compound excreted in the urine and feces 80% of the compound (i.e. 8.4% of the total 10.8%) was removed in urine and 75% (i.e. 45% of the 60%) of the compound was passed through the feces within the first 2 days thus indicating a rather efficient elimination.

It can therefore be concluded that under the test conditions the parent compound, fluvalinate, is poorly absorbed through the skin (also confirmed by acute dermal $LD_{50}$ studies) and that grooming can effectively result in considerable ingestion of compound.

Classification: Supplementary

Albin B. Kocialski

Albin Kocialski, Ph.D.
Toxicology Branch/HED (TS-769)

TS-796:th:TOX/HED:AKocialski:4-11-83:card misc. #27

Subject: Fluvalinate Skin Irritation Tests (Note: A Series of Tests Conducted on Skin).

Test Compound: Fluvalinate
Purity: 93.1%

Test Compound Source: Agricultural Chemicals Experimental Group of Mitsubishi (Japan)

Accession Number: 249604

Testing Facility: Mitsubishi - Kasei Institute of Toxicological and Environmental Sciences (Japan)

Report Number: MITES Report M56-266 (written in Japanese) translated into English and confirmed for accuracy by Japanese.

Testing Period: January - July 1982

Report Submitted to Sponosor: ca. February 10, 1983

Studies Conducted:

1. Primary skin irritation experiments
   - i. Using rats
   - ii. Using rabbits
2. Repeated dermal application experiments
   - i. Using acetone solution (includes pathological examinations)
   - ii. Using olive oil solution
3. Intracutaneous administration experiments
4. Subcutaneous administration experiments
5. Phototoxicity experiments

Experimental Animals:

Male and female SPF rats (Wistar or SD) strain obtained from the Shizuoka Prefecture Experimental Animals Agricultural Cooperative Association or Japan-Charles River. Age: 5-7 weeks. Body Weight: 109-117 grams for females, 141-276 grams for males. Quarantine Period: Approximately 1.0 week.

Male Rabbits: Supplier, Ishikawaya.
Body Weight: 2.0 - 2.4 kg.
Quarantine Period: 1.0 week

Housing All Animals: Temperature 22-24°C. Humidity: 50-60% for entire experimental period including quarantine. The rats were housed in wire mesh cages or polycarbonate cages (Model Fc-200; Tokiwa Scientific) in which there were metal platforms (flat top type). During quarantine, 5 animals were housed per cage. During the experimental period, 1 animal was housed per cage. (However, in the continuous application experiments using 15% acetone solution 5 animals were kept per cage at first, with 1 animal per cage from the 5th day.)

The rats were allowed to consume feed and water freely. The feed consisted of solid feed (MF: Oriental Yeast Industrial Co., Ltd.). The drinking water was tap water that had been sterilized by ultraviolet irradiation and which was supplied by an automatic water feeder or a water bottle.

The rabbits were housed in individual cages and feed was dispensed to them by automatic feeders (Tokiwa Scientific). The feed used was RC-4 rabbit solid feed (Oriential Yeast) and the water used was tap water that had been sterilized by ultraviolet irradiation and which was applied by an automatic water feeder. They were given free access to both feed and drinking water.

Primary Skin Irritation Experiments - Rats: Five 7 week old male SD strain rats (SPF). Weight was greater than 250 gms.

Experimental Design:

Fluvalinate was diluted to 15% with acetone and applied in a volume of 0.5 mls to an area 4 x 5 cm. on the back of the rat. The area of exposure had previously been shaved with hair clippers the day before followed by use of a depilatory agent. An occlusive patch was applied for 24 hours after which time the remaining test material was wiped off. Observations were made and scored according to the method of Draize for 7 days.

Results:

It was reported that no distinct abrasions were observed and that all animals gained weight. Signs observed were crouching, prostration, chromodacryorrhea, dacryohemorrhea, nose bleed and protrusion of the eyeballs. Some signs were attributed to the

effects of occlusive patching. Signs of primary skin irritation were absent by the fouth day. The maximum primary irritation score was 2.

Conclusion:

Based on the Draize method of scoring the compound can be classified a mild skin irritant.

Classification: Supplementary.

Rabbits: Two male albino rabbits were used.

Experimental Design:

The back of the rabbit was shaved with electric shears one day before administration of the test material. One area consisted of intact skin and one area was abraded (one rabbit only) with a 21 guage needle deep enough to penetrate the stratum corneum but not the dermis. A volume of 0.5 mls was then applied to each area. A gauze patch was then applied and held in place by a polyethylene sheet taped to the animal for a 24 hour period. The application site was then wiped clean with absorbent cotton soaked in water. Evaluation was then carried out by the method of Draize at 24 and 48 hours.

Results and Conclusions:

Based on the Draize method of scoring the compound can be considered a mild irritant.

Classification: Supplementary.

Repeated Dermal Appication (Acetone Vehicle) Experiment:

Experimental Design:

Five week old male SD strain rats (SPF) were used in the abraded group. Six to 7 week old male Wistar rats (SPF) were used with all other groups.

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Fluvalinate was diluted with acetone to concentrations of 15, 1.5, 0.3 and 0.15% (w/v). A volume of 0.05 ml per day of these concentrations was applied to the dosal cervical area (an area not reached by foot or mouth) with a ball tipped needle. With concentrations other than 15%, a control application with acetone only was applied in the lumbar region. In cases in which the 15% solution was used the region of application was shorn with hair clippers and chemically depilated. In the other groups shearing was performed with scissors and depilation was not informed. With the 15% solution applications were made to the intact group and the abraded group (abraded group; skin penetrated with a 21 G needle sufficient to penetrate the stratum corneum but not the dermis).

Number of daily treatments and order of performance of experiments.

| | | | | |
|---|---|---|---|---|
| 1. | 15% | (10)* | - | Intact Group -- 14 days, sacrifice on 15th day. |
| | | ( 3) | - | Abraded Group -- 7 days, sacrifice on 8th day |
| 2. | 1.5% | ( 5) | - | 5 days, sacrifice on 6th day (intact skin) |
| 3. | 0.3% | ( 5) | - | Same as #2 above |
| 4. | 0.15 | ( 5) | - | Same as #2 above |

* ( ) = Number of animals.

Pathological Examination:

Skin samples from all high dose group animals were excised, processed, sectioned, stained with hematoxylin and eosin, and examined under the micorscope.

Results:

General Observation:

Scrathing was observed in all groups approximately 15-20 minutes after application beginning about the second or third day. The day scrathing was first observed in the low dose group was not recorded.

Gross Pathology: 15% Intact Group: It was reported that petechiae were observed at the margins of the region of application in 5 cases from the third day of application. Similar petechiae and local ulceration in the margins as well as erosion were seen in 2 cases. Subsequently it was reported that these signs appeared in a repeated course of hemorrhage to ulceration to scabbing to hemorrhage. It was reported that this pattern

continued until the experiment was concluded. Decreases in spontaneous movement were observed in all cases. Housing arrangements were changed from 5 animals/cage to 1 animal per cage from the 5th day of application in order to keep the animals from wounding each other. Hemorrhage marks appeared over wide areas on the upper forelegs and at the bases of the ears in several cases from the 7th day of application. The same signs appeared in these sites as in the region of application and persisted until termination of the experiment. Spontaneous movement continued to decrease with abnormalities in walking becoming manifest from the 11th day until the experiment was concluded.

15% Abraded Group: Petechiae marks were seen around the wound in 2 cases and were seen in all animals from the 4th day onward. Signs similar to those in the Intact Group appeared in a repeated pattern and persisted until the experiment was concluded.

1.5% and 0.3% Groups: Results in these two groups were generally similar. Petechiae were observed 3 days after application, hemorrhage and scabbing were also observed.

0.15%: Mild reddening was observed in all cases (seen also in all other groups). However, no other symptoms were observed.

Histopathology: 15% Group Only: Abnormalities were seen in both the Intact Group and the Abraded Group. Proliferative thickening and necrosis (scrabbing) of the prickle cell layer and cellular infiltration of the subcutaneous tissues were seen in the region of application in the Intact Group. In the Abraded Group edema was observed in the region of application in addition to the aforementioned findings. The incidence of pathological changes were clearly greater in the abraded group.

Summary:

All groups were observed scratching with the intensity being dose related. Scabs appeared at the two top dose levels as close to the application site as the hind foot could reach. Onset was dose related and ulceration was observed at the top dose. Erythema at the low dose and no scabbing was observed.

Conclusion:

These results lead the petitioner to propose that the small lesions observed in the rat chronic gavage study may be created by grooming at the site of dermal contamination.

Classification: Core-Supplementary.

Repeated Dermal Application (Olive Oil Vehicle) Experiment

A similar type of experiment previously conducted with a as vehicle was conducted using olive oil as a vehicle.

Summary:

No scratching or scabs were observed with 0.15 or 7.5% solutions. At 10 and 15% solutions, scratching was observed after 30 minutes and most animals had scabs as close to the application site as the hind food could reach.

Conclusion:

The results lead the petitioner to propose that the smal lesions observed in the rat chronic gavage study may be creat grooming at the site of dermal application.

Classification: Core-Supplementary.

Intracutaneous Administration Experiments

Experimental Animals: A total of 20 six week old male S Wistar rats were employed.

Experimental Design: Fluvalinate was diluted to concent of 10% and 15% with olive oil. Amounts of 0.025 mls were app dermally (d) on each of 2 days on the right side of 5 animals (flank area) while amounts of 0.05 mls were administered once only on the left side by intracutaneous (i) injection. The r 5 animals were treated with a single intracutaneous injection of 0.05 mls on the left side only.

| 10% | | 10% | 15% | | |
|---|---|---|---|---|---|
| i | d | i | i | d | i |
| 0.05 | 0.025 | 0.25 | 0.05 | 0.025 | 0.05 |
| 5 animals | | 5 animals | 5 animals | | 5 anima |

Results:

10% (5 Animals): Dermal application on the right and intracutaneous injection on the left. - Signs were generally similar on both sides of the animal. Mild reddening of the application area followed by evidence of scratch marks (abrasions) were noted. Ulcerations were observed in 3/5 animals receiving the compound by injection.

Intracutaneous injection (5 animals). - Signs were qualitatively and quantitatively similar as noted above.

15% (5 animals): Dermal application on the right side and intracutaneous injection on the left. - Mild reddening (dermal application) was observed followed by evidence of scratch marks. However, these lesions disappeared 5 days after final application. Slight reddening was seen at the sites of injection, followed by signs of abrasion and scabbing.

Intracutaneous injection only (5 animals): Signs in evidence generally paralled those presented above.

Summary and Conclusion:

Scratching of the region of application and the site of intracutaneous injection and their peripheries with the hind paw and the mouth was confirmed in both concentration groups. Hemorrhage marks abrasions and scabs were observed.

Classification: Core-Suppelementary.

Subcutaneous Administration Experiments

Experimental Animals: Five 6 week old SPF Wistar rats.

Experimental Design: Fluvalinate diluted with olive oil to a concentration of 15% (w/v) was administered by the same method as for intracutaneous administration. A volume of 0.025 ml was applied dermally one a day for a two day period to the right flanks of five animals while amounts of 0.05 ml were administered once only by subcutaneous injection into the left flank. Observations were made for 7 days.

Results:

Dermal Application: Scab formation was seen in 2 cases and disappeared prior to termination of the observation period.

Subcutaneous Injection: Mild reddening and scaling was observed but was reversed within 7 days. Generalized grooming with the hind paws and mouth was observed from 20 minutes after administration on day of administration. This was transient and not distinguishable from a response to a subcutaneous saline injection (reported later). Scratching was not specifically directed at the sites of subcutaneous injection and was not persistent. No abrasions were seen subsequently.

Summary:

Abrasions thought to be due to scratching were found in the region of topical application. Although transient generalized grooming of the sites of subcutaneous injection was seen on the day of administration, the behavior was not obvious or persistent, and was not distingiushable from normal grooming and did not lead to abrasions.

Classification: Core-Supplementary.

Phototoxicity Experiment:

Experimental Animals: A total of 17 six week old male SPF Wistar rats were used with 7 animals in the 1.0% group and 10 animals in the 0.7% group.

Experimental Design: Fluvalinate was diluted to concentration of 1.0% and 0.7% (w/v) in acetone. Areas 2x2 cm. in the dorsal cervical region were shorn and depilated in the same manner as in the repeated dermal application experiments. A volume of 0.05 ml was applied on a one time basis. Five animals were used as a control group. Two animals were used in the non-irradiated group and applications made without immobilizing the animals. In the irradiated group animals in the 0.7% solution group, 5 animals were used in the irradiated group and 5 animals were used in the non-irradiated group.

Animals receiving radiation treatment were irradiated at 30 minutes after application with UV light equivalent to $1.4 \times 10^8$ erg/cm$^2$ through a soda glass. Animals were kept at a distance of 10 cm from the light source.

Observations of the 1.0% group occurred up to 5 days after irradiation and up to 3 days in the 0.7% group.

Summary of Results:

Scratch wounds and ulceration thought to be due to scratching were observed in irradiated and non-irradiated groups. No clear differences in signs between groups were observed.

Classification: Supplementary.

Ancillary Study: Subcutaneous injection and dermal application.

Communication: From Japan by TELEX.

Objective: Confirmation of absolute lack of response in subcutaneous treatment.

Materials and Methods: Three groups of animals, Sprague-Dawley rats, six per group.

Material was fluvalinate 15% in olive oil.

Group 1: Skin painted on right side, no treatment on left side (flank).

Group 2: Intracutaneous injection on the right flank, no treatment on the left flank.

Group 3: Subcutaneous injection on the right side, no treatment on the left side.

Results:

On day next to treatment, 2/6 subcutaneous sites showed a light brown spot suggesting upper penetration of compound. Sites found scratched and became lesions. Consideration should be given to an upward leaking of compound at the injection site or less than desireable injection technique since the technician was not the same as was used in the previous experiment.

Classification: Supplementary.

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OPP:HED:TOX:A.KOCIALSKI:sb 4/15/83 X77395 Rm 820 #m25-D12

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NOTE TO THE READER: This study has previously been reviewed and classified. It is included here for the purpose of completeness. The study can be found in Assession Numbers 248458 or 249604.

Subject: Delayed Hypersensitivity Response Pilot Study in CD Rats Subchronically Topically Exposed to Half Resolved Fluvalinate

Test Compound: Half-Resolved ZR-2310 (ERRATA 3210) Technical Fluvalinate, Run 23.

Accession No: 249604

Testing Facility: Quintox, Inc.
Richmond, Virginia

Study No.: None given

Testing Period: April 28 - May 5, 1982

Report Submitted to Sponsor: June 10, 1982

Purity of Test Material: 93%

Batch or Lot No.: Run 23

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Study No.: None given

Testing Period: April 28 - May 5, 1982

Report Submitted to Sponsor: June 10, 1982

Purity of Test Material: 93%

Batch or Lot No.: Run 23

Objective of Study:

The purpose of this pilot study was to determine the feasibility of similarly testing animals currently on a chronic feeding study, for a delayed type hypersensitivity response to fluvalinate and to refine appropriate methodology.

Materials and Methods:

The delayed type hypersensitivity response (DHR) to fluvalinate was evaluated in male Sprague-Dawley derived (Charles River CD) rats previously used in an IRDC pilot topical administration study. The test material had been applied topically on the intact dorsal cervical region daily for a period of 19 days. Animals were not shaved prior to treatment. Group 1 was exposed to 0.25 mg/kg of fluvalinate dissolved in corn oil, Group 2 was treated with 0.25 mg/kg of fluvalinate dissolved in acetone. Group 3 received corn oil only at a dose volume of 1.0 ml/kg. Upon conclusion of IRDC's portion of the experiment, the male animals were air-freighted to Quintox Labs where a fourth group of animals comprised totally of females was added to the study. This latter group served as the naive control group. It was previously determined that the sex difference would not affect the results of the study.

Reception of animals was followed by a 6-day acclimation period. Animals were examined for general health and housed in individual cages. The right ear of all the animals received from IRDC had attached metal ear tags, and as a consequence the right ears were swollen and occasionally infected. Two

(2) animals were observed intially as having lesions which then disappeared during the acclimation period. Animals were allowed free access to tap water and standard Purina Certified Laboratory Chow #5002.

Animals were weighed and injected IP with 8 X $10^{-6}$ molar fluorodeoxyuridine (FuDR). FuDR inhibits the enzyme thymidylate synthetase thus allowing for a greater incorporation of $^{125}$I-5-iododeoxyuridine (IuDR) into monocytes. Thirty (30) minutes later each animal received 10 microcuries of $^{125}$I-5-IuDR by IV injection. Twenty-four (24) hours after injection, each animal was challenged with 25 mg/kg of fluvalinate applied in a volume of 0.05 ml (acetone or corn oil, respective to the original vehicle applied at IRDC) to the left ear. The challenge dose of fluvalinate was 100 times the daily dose previously administered topically at IRDC.

Twenty-four (24) hours post-challenge the animals were sacrificed with chloroform and the following tissues and organs were removed:

- o right ear (punch biopsy)
- o left ear (punch biopsy)
- o right cervical lymph nodes
- o left cervical lymph nodes
- o spleen

Each tissue/organ was weighed and placed in a capped test tube. Samples were then counted on a gamma counter for 2 minutes.

Data was analyzed using the appropriate statistical methods.

Results:

General Observations: Prior to challenging the left ear, only normal grooming was observed. However, all animals began scratching at their left ear (challenged ear) and the area directly below the ear within 15 to 20 minutes post-challenge. Scratching continued for approximately 1.5 hours after which time normal grooming was again observed.

Absolute Tissue and Organ Weights of Animals Challenged with Fluvalinate. All parameters had variances which were homogeneous with the exception of the right ears of animals fitted with a metal ear tag. The naive animals did not have metal ear tags nor were their ears inflamed. Body weight, left ear weight, left and right lymph node weight and spleen weights were not significantly different than corn oil vehicle

control. The right ear weights (punch biopsy) of the naive group (untagged ears) were significantly lower than that of the corn oil control group (tagged ears).

Absolute Radioactivity of Tissues and Organs. All groups had lower left ear counts than the corn oil controls but only the left ear counts in animals exposed to fluvalinate in corn oil were statistically lower than corn oil controls. All groups had higher spleen counts than the corn oil group and all were significant at the $P < 0.05$ level. Neither left nor right node counts for all the groups differed from the corn oil control.

Relative Tissue and Organ Weight Ratios. There was a wide range in the body weights of the animals within each group. Therefore, an attempt was made to normalize the data by calculating the ratio of tissue and organ weights of each animal to its body weight.

No difference was observed between the corn oil control group and the fluvalinate treatment groups.

Relative Radioactivity of the Tissues and Organs of Fluvalinate Challenged Animals. Left and right node count ratios showed no significant differences from the corn oil control group. Both fluvalinate treated groups had spleen count ratios significantly higher than the corn oil control. The naive group showed the greatest effect; however, the increase was not significant as determined by the Wilcoxon rank sum test. The left ear count ratios followed the same pattern as the relative weights. The left ear ratios of the naive group were significantly lower than the corn oil control, however no difference was observed between the corn oil control group and the fluvalinate treatment groups. While there were no differences between the IRDC animals with respect to the right (tagged) ear count ratios, the naive animal's right (untagged) ear count ratio was significantly lower.

Discussion:

A delayed type of hypersensitivity response can manifest itself in some of the following ways:

- o increase in the challenge ear weight
- o increase in the challenge ear radioactivity
- o increase in the size of the draining lymph node of the challenged ear

- o increase in the radioactivity of the drainage lymph node of the challenged ear
- o possible increase in spleen weight
- o possible increase in spleen radioactivity

The above noted changes were not observed in the probe study.

The left ear weights (challenged ear) of the fluvalinate treated animals showed no difference as to absolute or relative weight compared to the corn oil vehicle. Left lymph node weights of fluvalinate treated animals showed no size differences as either absolute and relative weights. Similarly the spleen weights, both as absolute and relative weights, were not different than the corn oil vehicle.

The left ear counts of the corn oil control group were higher than either of the fluvalinate treated groups or the naive group on an absolute count or relative count basis. The left node count showed no differences between groups. Spleen counts of the fluvalinate treated animals did show an increase in both absolute and relative activity compared to the corn oil group. However, since the naive animals also had a significant increase in the counts compared to the corn oil group the increased spleen radioactivity may be attributed to a non-specific effect or may represent an increase in dividing cells which occurred in response to the animals scratching.

Although there was no positive control, per se, in the study the results of the right ear parameters can be interpreted to show that the assay did detect monocyte influx.

All animals received from IRDC appeared to have some measurable amount of infection of the right ear due to the presence of the metal ear tags. The right ear biopsy weights of all IRDC animals, regardless of the test group, were similar in weight and radioactivity count whereas the naive group had far fewer counts present in their right ears. The same pattern was observed if one considers the relative weights and counts.

Since one would expect to see monocytes at the site of infection, it was not surprising that the right ears, those with metal tags, showed significant increases in weight and radioactivity compared to ears without tags. Therefore, one might consider the right ear data as an "indirect" positive control.

Conclusion:

The data from this study suggest that male CD rats, topically exposed to half-resolved fluvalinate when applied under the regimen established by IRDC, do not become sensitized to the compound, since challenge with the fluvalinate fails to elicit a delayed type hypersensitivity response. Since IRDC documented dermal lesions on most of these animals, and since they do not respond with a delayed hypersensitivity response upon challenge, it is highly probable that another mechanism causes dermal lesion production.

Due to the limited number of animals available from the chronic dietary studies and the high probability of negative results based on the data of this study, it was not recommended that a similar study be carried out in the chronically exposed animals.

Classification: Core - Supplementary

Footnote: Zoecon has indicated that in this and other short-term studies in which Zoecon has investigated dermal lesion etiology, Zoecon has confirmed that all rodents responded promptly to an adequate topical dose of fluvalinate by attempting to scratch the application site. The onset of behavior was the same in naive as in previously exposed animals and was sufficiently rapid as to exclude an immune mechanism. It was also dose related in term of the vigor with which the rats scratched. If an adequate topical dose was applied daily, the scratched skin becames abraded after 2 or 3 days; given continued daily application, a dermal ulceration may be produced by this continued scratching.

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2/22/83

#m23

NOTE: This data was generated by Zoecon Corp. and is to be filed with fluvalinate. Caswell No. 934

Subject: 5-Week Rat Dietary Subchronic Toxicity Study with Fenvalerate (NOTE: not fluvalinate) Technical.

Test Compound: Fenvalerate Technical. Purity: 95%

Accession No.: 249604

Testing Facility: International Research and Development Corp.

Study No.: 322-058

Testing Period: June 29 - August 3, 1982

Report Submitted to Sponsor: November 5, 1982

Batch or Lot # from Label on Container Provided by Zoecon:

Fenvalerate tech. SD 43775
Code 11-5-0-0
Lot 80115 15 gms. Lot#80115, 1/2/79
C&AH R&D log 541
(amber solid)

Objective:

The purpose of this study was to evaluate the toxicity of fenvalerate technical to rats upon repeated dietary administration and to evaluate the potential to produce skin lesions.

Species Selection:

Charles River CD® rats were previously used in other studies conducted at the lab with related test articles. It was selected for this study for comparison purposes.

Materials and Methods:

Twenty-one (21) male Charles River CD® rats were obtained from the Charles River Breeding Laboratories Inc., of Portage, Michigan. Rats were housed individually and food (certified Rodent Chow® #5002, Ralston Purina Co.) and water were available ad libitum. Animals were observed regularly for signs of overt toxicity and mortality. The condition of the animals haircoat was recorded daily. Animals were acclimated for 14 days to

laboratory conditions. Room environment was controlled for temperature, humidity and a 12 hour light/dark cycle. Following the acclimation period 15 males with no readily apparent physical abnormalities were randomly selected and assigned to the treatment group. Each animal had attached an ear tag inscribed with a unique identification number.

Clean, disposable plastic protective clothing was worn by the trained personnel who performed the technical functions of the study.

The test article was offered in the diet at concentrations estimated to achieve a dosage level of 50 mg. of a.i./kg/day (NOTE: based on comparison of molecular weight and isomeric composition, the equivalent dose of fenvalerate (unresolved) was determined to be 50 mg/kg/day when the dose of fluvalinate was 60 mg/kg/day). Test article incorporation into feed followed procedures previously described. An initial amount of compound was mixed with acetone and blended into 500 g of animal feed with a Hobart mixer. This pre-mix was then added to a sufficient amount of lab chow and blended in a Hobart mixer to obtain the desired concentration.

Animals were observed twice daily, seven days a week for signs of overt toxicity moribundity and mortality. Detailed physical examinations were recorded daily on a regular schedule for general appearance, behavior, signs of toxicity and for the appearance of skin lesions. A careful record of each lesion was kept indicating, location, size and severity. Lesions were categorized as follows:

Hair loss: Area void of hair, surface of skin exposed.

Abrasion: A moist area, void of hair, where part of the skin has been scraped away but where the involvement is confined to the surface.

Ulceration: A moist area, void of hair, where the skin involvement is not confined to the surface layer and where muscle tissue may be involved.

Scabbing: A dry, hard area on the surface of the skin void of hair.

Body weights and food consumption were measured and recorded weekly.

Results:

Survival was 100% for the study duration. Group mean body weight gain was approximately 93.15%. Groups mean food consumption in terms of grams/animal/day remained relatively constant for the 5 weeks. Grams of food consumed per/kg/day decreased (30%) with time as expected. Compound consumption remained constant for the 5 week period averaging about 51.0 mg of active ingredient/kg/day.

No pharmacotoxic signs were observed for appearance and behavior.

Skin lesions were noted in 11/15 animals. The type of lesion and the number of lesions observed for each category of description was as follows:

Hair loss: 9/15

Abrasion: 9/15

Ulceration: 1/15

Scabbing: 11/15

Lesions were observed in the upper body generally in the dorso, lateral, cranial and cervical areas.

It is also pointed out here that lesion regression was also observed in several animals other than the 3 specifically identified by the study sponsor.

It is noted here that lesions were categorized according to the definitions presented in the methods section. We point this out to avoid any confusion between the number of animals showing scabs (as scabs by nature are indicative of the presence of hair loss) and the number of animals showing hair loss.

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Conclusion:

Fenvalerate under test conditions, and with the specific sample of test article employed was shown to produce skin lesions in rats when rats were exposed to the test article incorporated in feed.

Classification: Supplementary

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